

**AMENDMENT TO THE SPECIFICATION**

Please replace the first full paragraph on page 14, with the following amended paragraph:

In some cases, however, it may be preferable to select for the expressed proteins prior to assay. For example, the proteins expressed by the expression system may include an affinity tag. The affinity tag allows for immobilization of expressed protein as a result of binding of the tag to its binding partner. In an embodiment, recombinant proteins are engineered to include a poly-his affinity tag (*e.g.* (His)<sub>6</sub>) (SED ID. NO: 1). Proteins expressing the poly-histidine tag can be immobilized by binding of the tag to metals, such as zinc, nickel, cobalt, or commercial metal preparations such as TALON, and the like. Alternatively, proteins expressing affinity tags may be immobilized by binding of the affinity tag to protein binding partners such as antibodies and the like. For example, proteins expressing the poly-his tag can also be immobilized by binding to antibodies that recognize poly-his. Thus, the binding surface of the array may include either a metal coating or antibody to poly-his. Alternative affinity tags which can be recognized by antibodies specific for the tag epitope include a nine amino acid epitope from the human *c-myc* protein; a twelve amino acid epitope from protein-C; hemagglutinin (HA), or FLAG 8 (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys; SEQ ID NO: 2).

Please replace the first full paragraph on page 32, with the following amended paragraph:

One detection system of particular interest is time-of-flight mass spectrometry (TOF-MS). Using parallel sampling techniques, time-of-flight mass spectrometry may be used for the detailed characterization of hundreds of molecules in a sample mixture at each discreet location within the array. Time-of-flight mass spectrometry based systems

enable extremely rapid analysis (microseconds to milliseconds instead of seconds for scanning MS ~~devises~~ devices) high levels of selectivity compared to other techniques with good sensitivity (better than one part per million, as opposed to one part per ten thousand ~~for~~ for scanning MS)[[.]]. As a mass spectroscopic technique, time-of-flight mass spectrometry provides molecular weight and structural information for identification of unknown samples.

Please replace the first full paragraph on page 34, with the following amended paragraph:

A preferred method to couple the microchip based separation device to a detection system is the use of an electrospray source that can be interfaced between the output of the separation channel on the chip and a detection system based on either an atmospheric pressure ionization or an evacuated TOF-MS. The separation method utilized with TOF-MS (and other detections systems described below) may comprise electrophoresis, preferably utilizing electrochromatography as a means to separate ions based on both adsorption as well as migration. Electrospray and capillary electrophoresis both require high voltages, so the system should decouple the fields necessary for good separation efficiency and electrospray. An external sprayer coupled to the microchip by a liquid junction using readily available fused silica tubing allows for a very simple chip design that can be made of but not limited to glass or polymer. This approach minimizes the dead volume of the system and also allows for adding proper solvents and additives for good electrospray behavior. Figure [[5]] 4, shows a possible layout for such an interface.

Please replace the paragraph beginning on the bottom of page 34 and ending on the top of page 35 with the following amended paragraph:

In an embodiment, an electrospray device provides a reproducible controllable, robust means of producing nanoelectrospray of liquid sample from a silicon microchip (e.g. ~~Cornell University Nanofabrication Facility, <http://www.cnf.cornell.edu/>~~). Thus, an electrospray device may be fabricated from a monolithic silicon substrate using reactive ion-etching and other standard semiconductor techniques. The electrospray device for MDS analysis of the biochips of the present invention produces a stable cone with an electrospray voltage less than 1000 V. Nozzles may be as small as 15 microns in diameter (~~Gary Schultz Cornell University, <http://www.cnf.cornell.edu/>~~). The electrospray device may be interfaced to a time-of-flight mass spectrometer using continuous infusion of test compounds at the flow of rates less than 100 nL/min. Using such a system, a stable nanoelectrospray from a 20 micron diameter nozzle at 700V and 100n L/min of reserpine solution at 500ng/ml in 50% water/50% methanol solution can be generated (~~Gary Schultz Cornell University, <http://www.cnf.cornell.edu/>~~). For example, electrospray device lifetimes achieved thus far have exceeded 1 hr of continuous operation, a level which is sufficient for typical chip-based separations. Total volumes of less than 100 pL electrospray can be employed, a level which is suitable for combination with microfluidic separation devices.

Please delete the first full paragraph on page 35 and replace it with the following amended paragraph:

The performance of this electrospray device is equivalent to conventional nanoelectrospray (nL electrospray) using a tapered fused-silica capillary. The electrospray device may be positioned up to 10 mm from the orifice of a TOF-MS to establish a stable nanoelectrospray. Figure 4, shows a sketch of an electrospray device used for the arrays of the present invention. For example, a mass spectrum generated from the infusion of 1 mg/mL reserpine solution demonstrates a signal to noise ~~ratio~~ ratio of greater than 100, using a microchip-based electrospray device. (~~Gary~~ SchultzCornell University, <http://www.cnf.cornell.edu/>)

Please delete the full paragraph beginning on the bottom of page 37 and ending on the top of page 38 and replace it with the following amended paragraph:

To generate a library of related protein fragments, open reading frames of known protein targets identified in DNA databases are amplified by the polymerase chain reaction (PCR) for subcloning. For example, a receptor protein, enzyme binding domain, or enzyme catalytic site can be analyzed by computerized analysis for aspects of protein structure or function that are of interest. Programs used for proteomics analysis are well known in the art and include GCG (Genetics Computer Group; Madison, WI) and BLAST (~~see e.g. <http://www.ncbi.nlm.nih.gov>~~), Pfam-HMM, ScanProsite, SMART, CD-Search, SIM (~~see e.g. <http://www.ExPASy>~~), and PeptideSearch (EMBL, Protein and Peptide Group). Proteins may be related based upon three dimensional structure analysis, amino acid analysis, functional domain, or upon known similarities of function. Also, proteins of the same family or from the same species may be used to generate the library.

Once sequences of interest are identified, primers which flank those sequences are synthesized and the intervening sequences amplified by RT-PCR.

Please delete the first full paragraph beginning on page 38 and replace it with the following amended paragraph:

For most applications, *in vivo* expression of proteins is employed. Thus, cDNAs or PCR products are cloned into a commercial expression vectors such as LRCX retroviral vector set (Retro-X system; Clontech, Palo Alto, CA), MSCV retroviral expression system (Clontech; Palo Alto, CA), a baculovirus expression system (pFastBac; Life Technologies), or mammalian expression vectors which provide epitope tagging (e.g. pHM6 or pVM6, Roche Molecular Biochemicals, Indianapolis, IN; pFLAG SEQ ID NO: 2, Sigma, St. Louis, MO).

Please delete the second full paragraph beginning on page 41 and replace it with the following amended paragraph:

For example, by including the sequence encoding specific residues, expressed proteins can be synthesized with a tag, such as His<sub>6</sub> (SEQ ID NO: 1)(six histidine residue epitope) by including the sequence (CAC)<sub>6</sub> (SEQ ID NO: 3) in the primer used for PCR or by using a vector which includes the tag (e.g. pHM6 or pVM6 epitope tagging vector; Roche Molecular Biologicals). Polyhistidine-tagged fusion proteins can be purified with TALON metal affinity resin (Clontech). Other tagging vectors which are commercially available include tags recognized by antibodies to the peptide tag. Antibody-binding tags include peptides derived from the human *c-myc* protein (nine amino acid epitope),

Protein-C (a twelve amino acid epitope from the heavy chain of human Protein-C), Hemagglutinin (HA), FLAG (8 amino acid) (SEQ ID NO: 2), and the like.

Please delete the full paragraph beginning at line 21 of page 41 and ending on the top of page 42 and replace it with the following amended paragraph:

In some applications, it is necessary to remove the tag. To provide for easy removal of the tag, expressed proteins may be generated to include protease-sensitive cleavage site such as thrombin recognition sequence (P4-P3-Pro-Arg (or Lys)•P1'-P2'; P2-Arg (or Lys)•P1' or enterokinase recognition sequence (Asp<sub>4</sub>-Lys•X) (SEQ ID NO: 4) adjacent to the tag. Protease sites may be engineered into a vector by PCR-based oligonucleotide mutagenesis, or added to the inserts by synthesizing primer with the sequence.

Please delete the Abstract and replace it with the following amended Abstract:

The present invention relates to the generation of an array of protein expression systems for parallel *in vitro* screening of small molecule libraries, protein or peptide libraries, or other protein-binding components. In an aspect, the invention provides a spatially defined array of protein expression systems, ~~comprising:~~ The expression system array may include (a) a substrate; (b) a binding surface which covers some or all of the substrate surface; and (c) a plurality of discrete protein expression systems arranged in discrete positions on portions of ~~said the~~ the substrate covered by said binding surface. Also described are ~~method~~ methods of using the array for the rapid identification of compounds of able to interact with proteins expressed by any given array.